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Abstract

Increased pressure pain sensitivity and impaired descending pain control have been associated with chronic pain, but knowledge on the variability in the adult general population is lacking.

Pressure pain thresholds (PPTs) and descending pain control as assessed by conditioning pain modulation (CPM) were recorded in a randomly selected sample (n=2199, 53% females) of the Danish adult general population aged 18-70 years. PPTs were recorded over the tibialis anterior muscle and the upper trapezius muscle. CPM was defined as the difference between PPT assessments before and during conditioning with cold pressor pain (hand) for 2 min. Conditioning pain intensity was assessed on a visual analogue scale (VAS) and questionnaire data was collected.

Female sex ($P<0.001$) and younger age ($P\leq0.02$) was associated with lower PPTs at both body sites. For the trapezius muscle, high perceived stress were associated with lower PPTs ($P<0.02$), whereas an interaction was found between body mass index and sex. CPM potency was lower in females compared with males ($P\leq0.003$) whereas no association with age was found. Higher education ($P\leq0.05$), premature withdrawal from the cold pressor test ($P\leq0.02$) and high VAS score ($P\leq0.02$) were associated with a larger CPM response.

Perspectives

Data from this large population-based study provides new insight into the gender and age variation in pain sensitivity and CPM response. Decreased CPM potency and increased pain sensitivity in females were found, emphasizing the need to improve the understanding of its clinical consequences.

Introduction

Pain sensitivity and the function of descending pain control have been extensively studied in clinical populations [28, 43], but the variability in these pain measures using data from a large epidemiological study of the adult general population has not been published previously.

The status of specific pain mechanisms can be assessed experimentally by standardized activation of different pathways in the nociceptive system and quantitative assessment of the evoked responses [1, 2, 5, 53]. The conditioned pain modulation paradigm (CPM) is believed to reflect the net sum of descending pain inhibition and facilitation [47]. Assessment typically involves application of a conditioning tonic pain stimulus and probing the effect with a painful phasic test stimuli applied to an extra-segmental site [54]. Although CPM protocols vary across studies, consistent findings have shown decreased potency in chronic pain patients [17, 28, 31], but so far little is known about the variation of CPM in an adult general population. A systematic review based on 17 studies including 670 healthy participants in the reproductive age found that females show less efficient CPM compared with males, which has been suggested as an important factor in the higher prevalence of chronic pain found in females [37]. So far the difference in CPM between males and females has not been studied across the age span. Greater sensitivity to pressure pain stimuli has also been consistently observed in females compared with males [39], but the link between increased baseline pain sensitivity in females and the less efficient CPM response has so far not been examined [37]. While inconsistent results on the association between age and pain sensitivity have been reported [11, 14, 19] , previous findings on CPM efficacy in selected healthy adults suggest that there is an age related decline [12, 50] starting at middle-age (40-55 years) [24]. Studies examining the association between CPM potency, pain sensitivity and other health related factors that may influence the pain response have so far been inconclusive. Accumulating evidence point to an association between increased body mass index (BMI) and chronic pain [21, 32, 36, 51] and an influence on pain thresholds

has also been reported [38]. Sensitization of the nociceptive system due to systemic inflammation caused by fat tissue has been suggested as one possible explanation for this relationship [36]. BMI has so far not been examined in relation to CPM potency. Psychology has also been shown to influence the response to experimental pain [23, 44]. However, in relation to CPM a recent meta-analysis examining the potential confounding effects of psychological factors, e.g. perceived stress, concluded that results are inconclusive and that more research is needed [30]. Findings on the relation between the duration of the conditioning stimulus and CPM magnitude have so far been inconsistent in healthy subjects [15, 26, 40]. The influence of educational level on CPM and pain sensitivity has not been studied, but since the experience of pain is influenced by cognitively driven, supra spinal mechanisms the examination of a possible association is relevant [5, 56]. Moreover, an association between socio-economic factors and chronic pain is well established [10, 20]. Using an epidemiological approach, the primary purpose of the present study was thus to examine the sex and age related variations in CPM potency and pain sensitivity in the adult general population and secondly to examine the associations with BMI, level of perceived stress within the past month and educational level.

Methods

Study population

The present study is based on the first 2199 participants in the Danish Study of Functional Disorders (DanFunD). All invited persons were randomly drawn from the Danish Civil Registration system (each citizen in Denmark has a unique personal registration number), were between 18 - 70 years of age, and living in 10 municipalities in the south-western part of suburban Copenhagen. Exclusion criteria were: Not born in Denmark, not being a Danish citizen or pregnancy. The study was initiated November 2012 and pain assessment of participants was terminated by the end of 2013. Altogether 7942 were invited and 2199 participated (27.7%). All participants were fasting at the time of testing, i.e. no food or drinks after 11 pm prior to the day of testing. If a participant was scheduled for a time after 12.30 pm, a small meal no later than 6 hours prior to testing was allowed. Demographic and questionnaire data (i.e. self-reported pain, educational level and perceived stress) were collected. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). Information on use of pain medication either prescribed or over-the-counter drugs were registered on the day of testing. All participants gave written informed consent before taking part in the study, which was approved by the ethics committee (H-3-2012-015) and performed according to the principles of the Helsinki declaration.

Protocol

Participants were asked to lie down on a bed in a quiet room with the head elevated. Verbal information about the pain testing procedure was provided by a member of the staff performing the test. Participants were informed about the purpose of the study, pain testing procedures, and were asked to pay full attention to the procedure during the entire test. All 6 staff members performing the pain tests had received formal training in the testing procedures, which have all been validated

in other settings with good to excellent reliability [6 27]. The test procedure was as follows: 1) PPTs were assessed over the tibialis anterior muscle, 10 cm distal to the apex patellae on the non-dominant side and over the upper trapezius muscle, 10 cm from the acromion in direct line with the neck at the non-dominant side, 2) two minutes of cold pressor stimulation, and 3) re-assessment of PPTs over the tibialis anterior muscle during the cold pressor test.

Pressure algometry

PPTs at both testing sites were assessed with a handheld pressure algometer (Somedic, Sweden) mounted with a 1 cm^2 probe. The rate of pressure increase was kept at approximately 30 kPa/s. Participants were instructed to press a handheld push button to stop the pressure stimulation when the pressure sensation became painful, which defined the PPT. Participants were particularly instructed not to attempt to endure the pain as the test was a threshold and not a tolerance measure. A training procedure was applied in order to familiarize the participants with the assessment conditions. After the training, three PPT assessments were completed with 20 s intervals, never applying the algometer on the same skin spot although in close proximity. The mean value of the three PPTs recordings defined the PPT for further analysis.

Cold pressor stimulation and conditioned pain modulation

The participants immersed their dominant hand (to the wrist) in a circulating water bath at a cold temperature (maximum 3 °C) for 2 minutes. The water temperature was checked prior to each test to ensure that it was in accordance with the protocol. PPTs on the tibialis anterior muscle were reassessed (mean of three) after 1 minute of hand immersion following the same procedure as at baseline. If the conditioning pain stimulus became intolerable, the participant could terminate the stimulation earlier than scheduled. Participants were instructed to give notice before withdrawing

the hand so an assessment of PPT could be performed. The premature withdrawal in some individuals implied that PPT measurement was initiated before 1 minute of hand immersion. Data was still included in the statistical analyses in case only one or two of the three PPT recordings were obtained. Changes in PPTs from baseline to the reassessments during the conditioning cold-pressor stimulation were considered to reflect the CPM effect. The CPM effect was determined both as the absolute and percentage change as recommended by Yarnitsky and colleagues [53, 54]. After withdrawing the hand from the water bath, participants rated the intensity of the pain experience on a Visual Analogue Scale (VAS) where a score of 0 cm reflected "no pain" and a score of 10 cm reflected "worst pain imaginable".

Questionnaire data

Self-reported pain: Assessment of pain symptoms was based on a questionnaire about the presence of pain from muscles or joints, back pain, pain in the extremities, headache, chest or stomach pain within the past 12 months. Degree of pain was rated on a 5-point Likert scale with the following categories: "*not at all*", "*a little", "some", "a great deal" and "very much*". Scores were summarized into an ordinal scale ranging from 0 (no pain) to 28.

Educational level: Level of education was classified into 4 groups and defined as: 1) Skilled worker or less than 1 year of higher education, 2) less than 3 years of higher education, 3) 3 or 4 years of higher education, and 4) more than 4 years higher education.

Perceived stress: The Perceived Stress Scale (PSS) contains questions about thoughts and feelings during the past month and measures the degree to which situations in a person's life are appraised as stressful [7]. The PSS has been adapted to a short version consisting of 10 questions, the PSS-10, which has proven to be a valid and reliable measure of perceived stress [8]. Responses on the PSS-10 are rated on a 5-point Likert scale with the following categories: *"never", "almost never",*

"sometimes", "fairly often", and "very often". If more than 5 items were missing, the score was not included in the statistical analyses. The Danish translation has been validated by back translation to the original language and approval by the developer of the scale [33].

Statistics

Statistical analyses were performed using SPSS for Windows (version 22). Skewness and kurtosis and visual inspection of Normal Q-Q plots were used to inspect normality. Descriptive statistics are presented as mean and standard deviations (SD) or median and interquartile range (IQR) depending on the distribution of the continuous variables or as frequencies for categorical variables. Independent samples t-test, Pearson Chi-square, and Mann-Whitney U tests were used to examine differences in the distribution of age, BMI, perceived stress, educational level, self-reported pain symptoms (Likert scale) and use of pain medication (yes/no) between males and females. Level of significance was set at $P < 0.05$.

Baseline PPTs: With the purpose of testing the associations between PPTs at the tibialis anterior muscle and upper trapezius muscle and sex, age, BMI, perceived stress and educational level, multiple linear regression analyses were performed with PPTs as the dependent variables and the remaining variables as the explanatory variables. Associations of PPTs with sex and age, respectively, were examined in the first model adjusting for self-reported pain and use of pain medication. Next, BMI, perceived stress and educational level were analyzed in separate regression models adjusting for sex, age, self-reported pain and use of pain medication. Due to non-normal distributions, the PPT variables were log transformed prior to statistical analysis. The back transformed estimates and 95% confidence interval (CI) are presented. Initial analyses showed a quadratic rather than linear relationship between PPTs and age ($P < 0.01$), and age was thus categorized into four groups (18-39, 40-49, 50-59, 60-70 years). The same applied to the

relationship between PPT at the upper trapezius muscle and BMI, and the BMI variable was thus categorized according to the WHO recommendations [9]. Since the group with a BMI less than 18.5 kg/m^2 was rather small (N = 60), this group was merged with the normal weight group (BMI: 18.50 - 24.99 kg/m^2).

CPM effect: To analyze the association between the CPM effect, sex and age a multiple linear regression analysis was applied with the CPM effect as the dependent variable and sex and age as the explanatory variables. Since the CPM effect is relative to the baseline PPT value, controlling for baseline PPT value is relevant when examining the association between CPM and other factors. Associations with BMI, educational level, perceived stress, VAS score and duration of the cold pressor test were subsequently analyzed in separate linear regression models after adjusting for baseline PPT, sex, age, self-reported pain and use of pain medication. A total of 748 (38.9%) completed the cold pressor test for 2 min and the variable was thus categorized into 3 groups for the statistical analyses: 1) completion of 2 min, 2) completion between 1 min and less than 2 min, and 3) completion of less than 1 min. Subsequent sensitivity analyses were performed for the cold pressor test variable including sex, age, baseline PPT, self-reported pain and pain medication use in the model.

To check if inclusion of data based on less than 3 PPT assessments influenced the CPM findings, a control analysis on sex and age adjusted for baseline PPT, self-reported pain and medication use was performed including only participants with triple PPT recordings.

Results

Demographic characteristics

Compared to non-respondents, more females than males took part in the study ($P < 0.001$) and mean age was higher in respondents (50.7 (sd 13.5) years) compared to non-respondents (44.7 (sd 15.9) years ($P < 0.001$)). In this study, more females than males participated whereas no difference in mean age was observed between sexes (Table 1). Self-reported pain and use of pain medication were more frequent in females than in males. The same applied to level of perceived stress that was higher in females, whereas males had significantly higher BMI and levels of education (Table 1). Figure 1 shows the number of participants for each pain assessment procedure.

Baseline pressure pain sensitivity

The tibialis anterior muscle: The median (IQR) PPT at the tibialis anterior muscle in the total population was 512.0 kPa (329.3 kPa). Examining baseline PPT in participants with only complete PPT measurements (i.e. 3 assessments) did not change the results (median (IQR): 512.0 kPa (329.0 kPa)). Descriptive statistics of baseline PPT in males and females across age groups, BMI and educational level are presented in Table 2. The linear regression analysis demonstrated that compared with males, females had lower PPTs and the youngest age group had lower PPTs compared with the age groups above 40 years (Table 3). No associations were found with BMI, perceived stress or educational level (Table 3).

The upper trapezius muscle: The median (IQR) PPT at the upper trapezius muscle in the total population was 446.3 kPa (275.3 kPa). Examining baseline PPT in participants with only complete PPT measurements (i.e. 3 assessments) did not change the results (median (IQR): 446.0 kPa (275.0

kPa)). The linear regression analysis showed that females had lower PPTs compared with males, and the younger age groups had lower PPTs when compared to the older age groups above 50 years (Table 3). A significant interaction was found between sex and BMI suggesting that the effect of sex on PPT differed across BMI levels. High perceived stress was associated with lower PPTs whereas no association with educational level was found (Table 3).

Conditioning pain modulation

In the total sample the duration (median/IQR) of the conditioning cold pressor stimuli was 106.0 (65.0) s; 117.0 (40.5) s in males compared to 98.0 (70.0) s in females ($P < 0.001$, Mann-Whitney U Test). The conditioning VAS score was (median/IQR) 7.0 (3.0) cm in the total sample, and 6.0 (3.0) cm in males compared to 7.0 (3.0) cm in females ($P < 0.001$, Mann-Whitney U Test).

Scatterplots of raw PPT and CPM data in males and females across age groups are shown in Figure 2. In the total population, the absolute CPM effect was (median (IQR)) 178.0 kPa (190.1 kPa) and the relative increase was 35.9 % (43.2 %). Descriptive statistics of the CPM in males and females across age groups, educational level and BMI are shown in Table 4. The CPM effect was larger in males than in females after adjusting for baseline PPT both in absolute terms (Table 5) and in relative terms (Table 6). No significant linear relationship between the CPM effect and age was found (Table 5 and Table 6). Including age as a continuous variable in the model (absolute CPM) instead of the categorical variable did not change the result ($P = 0.69$). No associations with BMI or perceived stress was found, whereas a positive linear relationship (i.e. a large CPM effect) with increasing educational levels was found although only significant when having 3 or 4 years of higher education. A significant positive linear relationship with increasing cold pressor VAS score was found. Completing less than 2 min of the cold pressor test was also associated with a higher

absolute CPM response (Table 5), whereas the same applied for completing less than 1 min for percent CPM.

Performing sensitivity analyses in relation to the cold pressor test including only the participants who completed the per protocol 2 min of cold pressor stimulation (n = 772) and the participants who completed less than 1 min (n= 477) showed that in the group that completed the 2 min with cold pressor test, the absolute ($P = 0.11$) and percent difference ($P = 0.28$) in CPM between males and females was no longer significant, whereas the results for age remained unchanged ($P \geq 0.10$). In the group that completed less than 1 min, a significant effect of sex was confirmed with the magnitude of the CPM response being smaller in females compared with males (absolute CPM: $P < 0.001$, percent CPM: $P < 0.001$), whereas no significant effect of age was found ($P \geq 0.61$). Examining the distribution between males and females in the two groups showed a significant difference with a preponderance of males completing the 2 min compared with females, and a preponderance of females in the group completing less than 1 min ($P < 0.001$).

Performing control analyses on sex and age including only participants with three complete PPT assessments in relation to the cold pressor test (n = 1227) showed the same tendencies (Sex: $P = 0.02$ (absolute CPM) and 0.045 (percent CPM), Age: $P \geq 0.34$ (absolute CPM) and $P \geq 0.34$ (percent CPM) as reported in Table 5 and 6, i.e. the CPM response was larger in males compared with females and there was no association with age.

Discussion

Data from this large, randomly selected sample of the adult general population confirmed that females were more sensitive to pressure pain as compared with males and had reduced CPM potency. Younger age was associated with being more sensitive to pressure pain, whereas no association between age and CPM was shown. High perceived stress was associated with lower trapezius PPTs, but not with PPTs at the tibialis or CPM. The same applied to BMI, where an interaction with sex was found. No associations between educational level and pressure pain sensitivity was found, whereas having 3 or 4 years of higher education was associated with a larger CPM response. Premature withdrawal from the cold pressor test and high VAS score were both associated with a larger CPM response.

Pressure pain sensitivity

Previous findings on the association between sex and pain sensitivity in nonclinical populations have been inconsistent, which may in part be explained by the heterogeneity of available methods for evaluating the pain response, study populations and insufficient statistical power in some studies [39]. However, the present data support the conclusions based on systematic reviews that point to greater pain sensitivity in females compared with males and especially to pressure pain assessments [3, 29, 39]. The factors, whether physiological, psychological or psychosocial or an interplay, responsible for this difference have so far not been fully clarified [3, 20], although some studies suggest that gender-specific psychological factors influence the response to pain and thus offer some explanation to the observed male-female differences [23, 44].

The results also support existing studies on age and PPTs pointing to increased thresholds with advancing age [22, 24, 25]. For example, Jensen and colleagues examined PPTs in a random sample of

1000 adults aged between 25 - 64 years [22], and found significantly increasing thresholds with advancing age. Lariviére and colleagues [24] examined 60 healthy adults and likewise found lower PPTs in the youngest participants compared to both middle-aged and older participants. Some authors have suggested that findings of age differences in pain thresholds may depend on stimulus duration and that a longer response time in older people may account for the differences [19]. Although results from this study is limited to PPT assessments, no differences in PPTs between the age groups above 40 years for assessments on the tibialis anterior muscle and above 50 years for assessments on the upper trapezius muscle were found suggesting that a longer response time in the elderly have not influenced the findings.

High perceived stress was associated with lower PPTs, but only at the upper trapezius muscle. Increased sensitivity to pressure pain at the trapezius muscle has been reported in a study comparing persons on sick leave due to stress with healthy controls [18], and it is thus likely that high levels of perceived stress may contribute to increased pain in this particular body area [52]. Being obese or overweight was associated with higher PPTs (i.e. less pain sensitivity) at the upper trapezius muscle whereas no association was found for the tibialis anterior muscle. However, a significant interaction between sex and BMI was found for the upper trapezius muscle suggesting that the strength of the linear relationship across BMI levels differ between males and females. A recent systematic review concluded that existing studies do not clearly demonstrate that body weight influence pain perception [45]. One study has reported site-specific differences in pain sensitivity in obese and non-obese persons between 18 and 45 years of age with the obese being less sensitive, but only in areas with excess subcutaneous fat, e.g. the abdomen [38]. In support of this conclusion, the study found no differences in central pain processing, i.e. temporal pain summation and CPM [38]. In line, no association between BMI and CPM was found in the present study. Nevertheless the present data altogether suggests that the upper trapezius is a body area more sensitive to pain stimulation than

the tibialis anterior as shown by the lower overall thresholds levels in both males and females and the influence of perceived stress and BMI.

Conditioned pain modulation

Significant differences in CPM potency were found between males and females with the response being smaller in females after adjusting for baseline pain sensitivity, self-reported pain and use of pain medication. Inconsistent findings on sex-related differences in CPM have been reported in more studies with some reporting no observed difference [34, 35, 46, 49]. However, a systematic review concluded that existing data support a decreased CPM response in females compared with males, and in particular in studies using pressure pain stimulation [37]. A recent study assessing relative CPM by utilizing pressure pain thresholds and a cold pressor test in a group of healthy university students further support this conclusion [4]. Though a major strength of this study is the large sample size, the difference between males and females was no longer significant when examining the subgroup that completed the per protocol 2 min. This finding could suggest that sensitivity to the cold pressor test constitute a bias when applied as a conditioning stimulus. Premature withdrawal from the cold pressor test and higher pain intensity scores were both associated with a larger CPM response, which additionally suggest that both sensitivity and the subjective intensity of the cold pressor test plays a role in the potency of the CPM response. A number of studies on the involvement of intensity and duration of the conditioning stimulus on CPM extent have been performed [15, 26, 40]. These studies suggest that the CPM response is not dependent on conditioning stimulus intensity, i.e. painful versus non-painful [26], pain intensity [15, 55] or duration [40]. While not the primary aim, others have reported that CPM effect do in fact depend on, e.g. the intensity of the conditioning stimuli [13]. Altogether, the present study support a male-female difference in CPM

potency, but this finding needs to be replicated in another epidemiological study applying a different CPM methodology before any firm conclusions can be drawn.

The effects of aging on the CPM response have been tested in healthy individuals in previous studies using different methodologies [12, 16, 24, 41, 42, 50]. Although findings from these studies are inconsistent and the study populations are generally small, the majority report an age-dependent decline in CPM and are, therefore, in contrast to the present study. However, this study is based on a large random sample of the general population and all analyses were adjusted for baseline PPT, self-reported pain and use of pain medication which supports the findings of no association between age and CPM magnitude. Nevertheless, further studies are needed to determine the role of age in CPM.

A significant association was found between CPM and educational level, i.e. the CPM effect was larger in participants with 3 or 4 years of higher education compared to being a skilled worker or having less than 1 year of higher education. No other significant associations were found and comparisons with other studies are not possible since no other studies have examined this relationship. Moreover, no associations between PPTs and education were found. However, while the effect of education was small in this study other research has shown that education may influence disease status [48] and further exploration is thus needed.

Strengths and limitations

A major strength of this study is the population-based design including a random sample of the adult general population, but some limitations must be considered when interpreting the findings. The study was conducted in a random sample of the Danish adult general population and the results may thus not necessarily be generalizable to other populations. The response proportion of 27% may be considered low and with a possible influence on the results because of selection bias.

Comparing respondents with non-respondents showed that females were more likely to participate in the study compared with males and older people more likely than younger, which may affect the generalizability of the results.

Self-reported pain and use of pain medication were included in the analyses as possible confounders. However, some limitations must be considered since the duration of self-reported pain, i.e. whether acute/subacute or chronic, could not be determined based on the pain questionnaire data used in the present study, which enquired about the presence and intensity of pain during the past 12 months. Furthermore, participants may have experienced pain from other parts of the body than the ones enquired about.

Altogether, only 39% of the study population completed the cold pressor test according to the protocol, which could suggest that the conditioning stimulus were perceived as strong by the majority. Although no specific CPM protocol has been recommended [54] reducing the time of the conditioning stimulus, i.e. the cold pressor test, or applying individually tailored temperature levels may improve compliance with the testing procedure and thus reduce the possibility of bias. However, variations in the ability or willingness to undergo a painful testing procedure are to be expected in a general population sample despite careful instructions from the staff performing the tests.

Conclusions

This study provides new knowledge on the gender and age variation in pain sensitivity and descending pain control by using data from a large and randomly selected adult population-based sample. The confirmation of decreased CPM potency and greater pain sensitivity in females compared with males emphasize the need to improve the understanding of the clinical consequences

in females and thus more research into the mechanisms responsible for the male-female difference. Younger age was associated with greater pain sensitivity, whereas an age-dependent decline in CPM potency could not be confirmed and age may thus not play a major role in the individual ability to modulate pain. The trapezius area was more sensitive to pressure pain than the tibialis and an association with high perceived stress was found, which suggests that psychological factors needs to be taken into account. Duration and intensity of the conditioning stimulus was associated with a larger CPM response suggesting that CPM is not entirely independent of methodology.

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Author contributions

SS, TJ, LAN, JFE, and TGN conceived and designed the study protocol for the present study, interpreted the data, and critically revised the manuscript. SS analyzed the data and drafted the manuscript. As PI and member, respectively, of the DanFunD steering committee TJ and SS designed and initiated the DanFunD study and JFE contributed to all parts of the process as well as being the daily project manager. TC contributed to the design of the DanFunD study and critically revised the manuscript. All authors discussed the results and commented on the manuscript and approved the final version.

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Figure 1: Participant flowchart for the pain testing sequence

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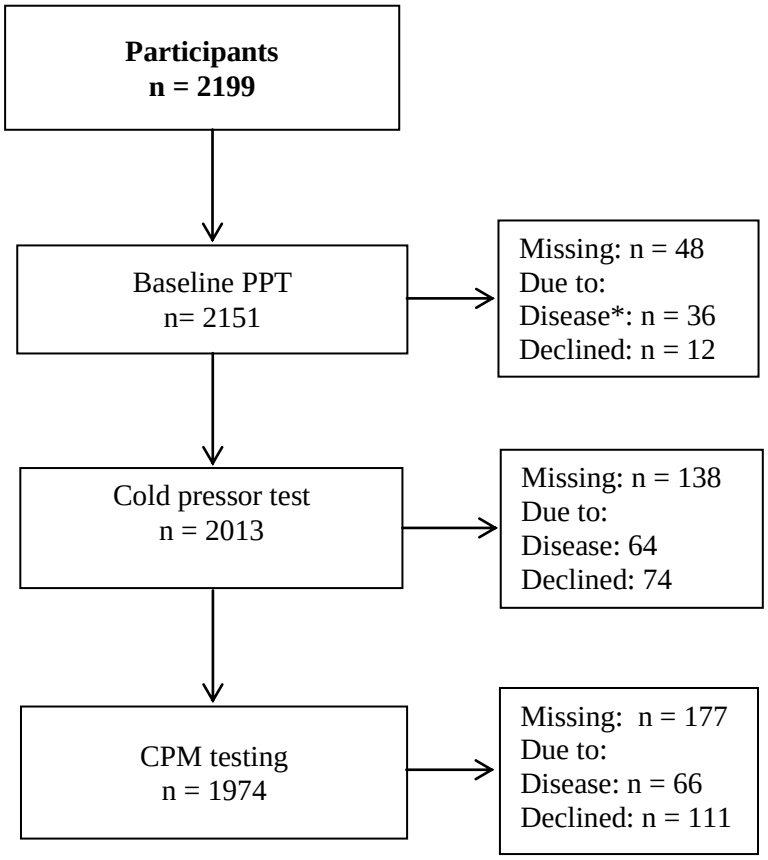
Figure

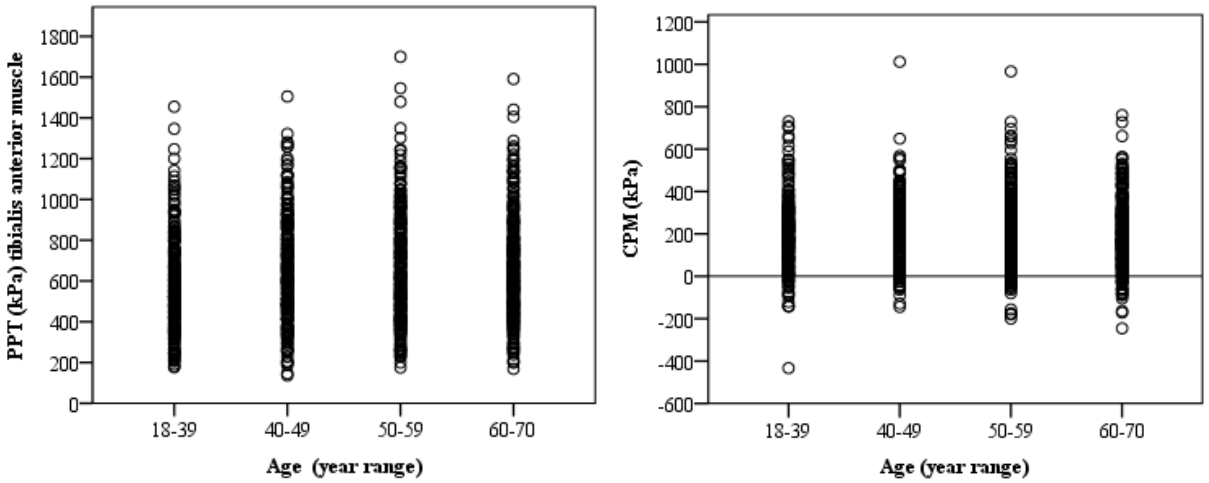
Figure 1. Participant flowchart for the pain testing sequence



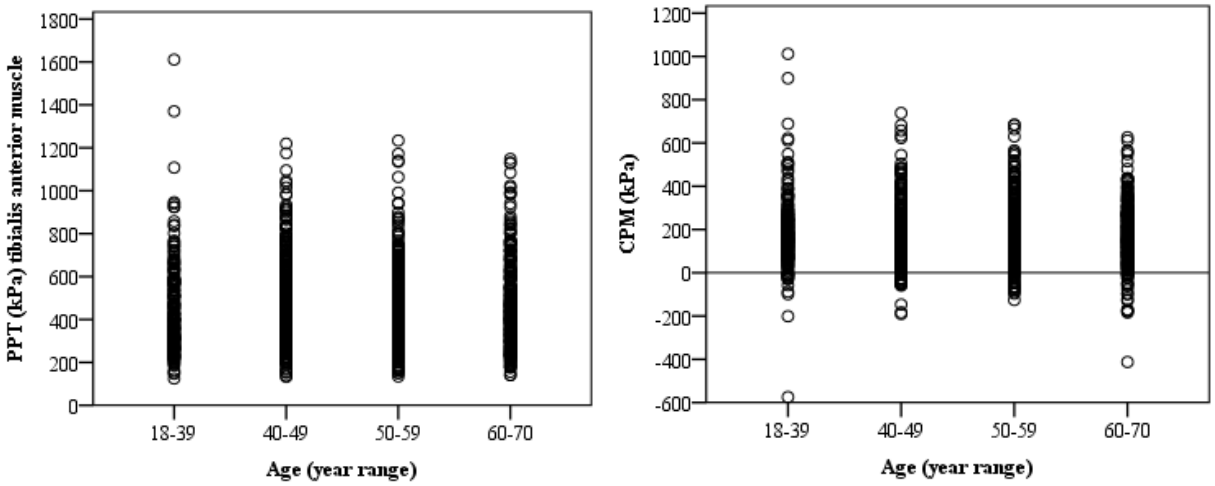
*Diseases where pain testing was contraindicated such as heart disease, crural edema or changes
to the skin due to treatment with corticosteroids.

Figure 2AB: Scatterplot of raw data on baseline PPT and CPM in males (A) and females (B)

A. Males



B. Females



Tables

Table 1: Sample characteristics

Variable	Total	Males	Females	P-value
N=	2199	1035 (46.8%)	1164 (52.6%)	
Age (mean (Sd)	50.7 (13.5)	50.9 (13.8)	50.6 (13.3)	0.63[1]
18-39 years (n (%))	465 (21.1)	227 (21.9)	238 (20.4)	
40-49 years	483 (22.0)	211 (20.4)	272 (23.4)	
50-59 years	595 (27.1)	278 (26.9)	317 (27.2)	
60-70 years	656 (29.8)	319 (30.8)	337 (29.0)	
BMI (n (%))				
Normal weight ≤ 24.99	1080 (49.1)	407 (39.3)	673 (57.8)	< 0.001[2]
Overweight ≥ 25.00	774 (35.2)	442 (42.7)	332 (28.5)	
Obese ≥ 30.00	344 (15.7)	185 (17.9)	159 (13.7)	
Perceived stress (mean((Sd)	10.4 (6.1)	9.3 (5.6)	11.4 (6.3)	< 0.001[1]
Educational level (n (%))				
Skilled worker or < 1 year higher education	673 (36.4)	336 (38.3)	337 (34.7)	< 0.001[2]
< 3 years higher education	401 (21.7)	178 (20.3)	223 (23.0)	
3 or 4 years higher education	548 (29.6)	234 (26.7)	314 (32.3)	
> 4 years higher education	227 (12.3)	130 (14.8)	97 (10.0)	
Pain symptoms (mean (Sd))	5.2 (4.1)	4.4 (3.6)	5.8 (4.4)	< 0.001[1]
Use of pain medication (yes (%))	56 (2.5%)	19 (1.8%)	37 (3.2)	0.047[2]

[1]Independent samples t-test
[2]Pearson Chi-square

Table 2: Descriptive statistics of PPTs across sex, age groups, BMI and educational level.

Total sample	**Tibialis anterior muscle N = 2151**		**Upper trapezius muscle N = 2152**	
Median (IQR)	512.0 (329.3)		446.3 (275.3)	
	Males	**Females**	**Males**	**Females**
	N = 1008	N = 1143	N = 1009	N = 1143
Median (IQR)	612.0 (347.4)	428.3 (272.3)	524.0 (295.2)	388.3 (239.3)
Age groups:				
18 - 39 years	545.3 (323.1)	389.0 (266.3)	425.2 (259.0)	333.7 (218.7)
40 - 49 years	639.0 (383.8)	475.8 (304.6)	524.2 (299.8)	397.3 (225.7)
50 - 59 years	631.7 (390.7)	425.3 (276.1)	558.3 (287.3)	386.7 (299.0)
60 -70 years	629.2 (311.1)	430.0 (242.1)	562.7 (299.3)	416.0 (247.0)
BMI:				
Normal weight ≤ 24.99	583.5 (347.8)	428.0 (280.3)	463.7 (255.4)	373.7 (225.5)
Overweight ≥ 25.00	642.7 (350.8)	440.8 (258.3)	562.7 (288.7)	407.8 (232.8)
Obese ≥ 30.00	606.3 (344.4)	406.7 (233.5)	573.0 (312.8)	408.5 (256.1)
Educational level:				
Skilled worker or < 1 year higher education	624.7 (351.7)	430.5 (250.1)	533.8 (331.4)	381.0 (226.0)
< 3 years higher education	594.5 (300.3)	424.3 (305.2)	516.5 (265.0)	379.7 (227.2)
3 or 4 years higher education	616.5 (365.5)	443.7 (304.0)	536.3 (287.6)	411.3 (257.2)
> 4 years higher education	635.3 (356.7)	415.7 (218.7)	540.3 (300.3)	379.0 (246.7)

Table 3: Multiple linear regression analyses with PPTs as the dependent variables and sex, age, BMI, perceived stress and educational level as the explanatory variables.

Variables	**Tibialis anterior muscle**		**Upper trapezius muscle**	
	B[1] (95% CI)	P-value[4]	B (95% CI)	P-value[5]
	N = 2134		N = 2135	
Sex				
Females	0.87 (0.85 - 088)	< **0.001**	0.89 (0.88 - 1.78)	< **0.001**
Males	-	-	-	-
Age groups[1]:				
18 - 39 years	0.95 (0.93 - 0.97)	< **0.001**	0.89 (0.88 - 0.91)	< **0.001**
40 - 49 years	1.01 (0.99 - 1.05)	0.21	0.97 (0.95 - 1.00)	**0.02**
50 - 59 years	1.00 (0.98 - 1.02)	0.99	0.88 (0.97 - 1.01)	0.23
60 - 70 years	-	-	-	-
	N = 2133		N = 2134	
BMI[3]**:**				
Obese ≥ 30 kg/m^2	0.99 (0.97 - 1.02)	0.06	1.04 (1.02 - 1.07)	< **0.001**
Overweight ≥ 25 - <30 kg/m^2	1.02 (1.00 - 1.05)	0.52	1.05 (1.03 - 1.07)	< **0.001**
Normal weight < 25 kg/m^2	-	-	-	-
	N = 2082		N = 2084	
Perceived stress[3]	0.999 (0.998 - 1.01)	0.72	0.998 (0.996 - 0.999)	**0.02**
	N = 1795		N = 1795	
Educational level[3]**:**				
> 4 years higher education	0.99 (0.97 - 1.03)	0.66	0.99 (0.96 - 1.02)	0.62
3 or 4 years higher education	1.01 (0.99 - 1.03)	0.26	1.01 (0.99 - 1.03)	0.35
< 3 years higher education	0.99 (0.96 - 1.01)	0.29	0.99 (0.97 - 1.02)	0.55
Skilled worker or < 1 year higher education	-	-	-	-

[1] Unstandardized coefficients.

[2]No significant interactions were found between sex and age for either the tibialis anterior muscle (P = 0.053) or the upper trapezius muscles (P= 0.12).

[3]Analyses of BMI, educational level and perceived stress were all adjusted for sex, age, self-reported pain and use of pain medication.

[4]No significant interactions were found between sex and: BMI (P = 0.09) , perceived stress (P = 0.68) or educational level (P = 0.72), or between age and: BMI (P = 0.41), perceived stress (P = 0.52) or educational level (P = 0.79).

[5] A significant interaction was found for sex and BMI (B 0.97 (95%CI 0.95 - 0.99) P < 0.01), but not with perceived stress (P = 0.26) or educational level (P =0.13), or between age and: BMI (P = 0.34), perceived stress (P = 0.69) or educational level (P = 0.85).

Table 4: Descriptive statistics of CPM across sex, age groups, BMI and educational level presented as medians (IQR).

Variables	**CPM N= 1974**			
	Absolute CPM		**Percent CPM**	
	Males[1]	**Females[2]**	**Males**	**Females**
	N = 926	N = 1048	N = 926	N = 1048
Total sample (Median (IQR))	197.3 (210.8)	166.00 (172.3)	32.1 (40.5)	39.4 (45.3)
Age groups:				
18 - 39 years	197.6 (206.3)	158.0 (151.5)	33.0 (41.1)	42.9 (47.5)
40 - 49 years	196.6 (216.0)	170.0 (169.1)	30.7 (38.8)	37.4 (41.1)
50 - 59 years	200.0 (228.1)	169.7 (187.6)	32.6 (42.0)	39.2 (43.8)
60 - 70 years	196.5 (198.9)	167.3 (181.6)	31.7 (41.2)	40.8 (48.1)
BMI:				
Normal weight ≤ 24.99	184.0 (209.8)	163.0 (160.2)	30.8 (40.4)	39.3 (45.1)
Overweight ≥ 25.00	208.0 (217.2)	164.0 (177.8)	33.9 (42.4)	38.7 (44.2)
Obese ≥ 30.00	200.3 (184.4)	183.8 (205.6)	34.6 (37.1)	42.2 (54.8)
Educational level:				
Skilled worker or < 1 year higher education	190.3 (235.7)	163.0 (175.0)	30.9 (45.1)	38.4 (45.3)
< 3 years higher education	198.3 (182.0)	154.3 (200.8)	34.3 (37.4)	38.5 (47.2)
3 or 4 years higher education	209.0 (224.3)	169.0 (164.0)	35.1 (43.1)	37.8 (43.2)
> 4 years higher education	199.2 (209.3)	201.0 (195.3)	29.9 (40.4)	50.1 (46.7)

[1]In males, baseline PPT (Median (IQR)) was 613.3 (349.3) and 824.0 (450.8) during cold pressor stimulation.

[2] In females, baseline PPT (Median (IQR)) was 428.8 (272.8) and 609.0 (344.8) during cold pressor stimulation.

Table 5: Multiple linear regression analyses with absolute CPM as the dependent variable and sex, age, BMI perceived stress, educational level, cold pressor test duration and VAS score as the explanatory variables.

Variables	**Absolute CPM**	
	B[1] (95% CI)	P-value[2]
	N = 1958	
Sex[3]		
Females	- 23.00 (-38.23 - -7.77)	**0.003**
Males	-	-
Age groups:		
18 - 39 years	1.44 (-18.66 - 21.53)	0.89
40 - 49 years	5.39 (-14.34 - 25.12)	0.59
50 - 59 years	10.24 (-8.69 - 29.17)	0.29
60 - 70 years	-	-
	N = 1957	
BMI[4]**:**		
Obese ≥ 30 kg/m^2	7.71 (-8.30 - 23.73)	0.35
Overweight ≥25 - < 30 kg/m^2	20.23 (-0.91 - 43.71)	0.06
Normal weight < 25 kg/m^2	-	-
	N = 1910	
Perceived stress[4]	- 0.69 (-1.98 - 0.59)	0.29
	N = 1655	
Educational level[4]**:**		
> 4 years higher education	22.01 (-3.39 - 47.70)	0.09
3 or 4 years higher education	22.15 (2.95 - 41.35)	**0.02**
< 3 years higher education	3.19 (-17.72 - 24.09)	0.77
Skilled worker or < 1 year higher education	-	-
	N = 1937	
Duration of the cold pressor test[4]**:**		
Completion of < 1 min	24.15 (4.23 - 44.06)	**0.02**
Completion of 1-2 min	20.81 (3.26 - 38.36)	**0.02**
Completion of 2 min	-	-
VAS score of the cold pressor test[4]	4.19 (0.76 - 7.63)	**0.02**

[1]Unstandardized coefficients.
[2] All analyses were adjusted for baseline PPT, self-reported pain and use of pain medication. In addition, analyses on BMI, educational level, perceived stress, cold pressor test duration and VAS score were adjusted for sex and age.
[3] No significant interactions were found between sex and age (P = 0.38).
[4] No significant interactions were found between sex and: BMI (P = 0.26), perceived stress (P = 0.77), educational level (P = 0.09), cold pressor test duration (P = 0.11) or cold pressor VAS score (P = 0.82).

Table 6: Multiple linear regression analyses with percent CPM as the dependent variable and sex, age, BMI perceived stress, educational level, cold pressor test duration and VAS score as the explanatory variables.

Variables	**Percent CPM**	
	B[1] (95% CI)	P-value[2]
	N = 1958	
Sex[3]		
Females	-5.36 (-8.53 - -2.18)	**0.001**
Males	-	-
Age groups:		
18 - 39 years	-0.42 (- 4.60 - 3.77)	0.85
40 - 49 years	0.38 (-3.73 - 4.49)	0.86
50 - 59 years	0.78 (-3.16 - 4.73)	0.69
60 - 70 years	-	-
	N = 1957	
BMI[4]**:**		
Obese ≥ 30 kg/m^2	3.83 (-0.57 - 8.23)	0.09
Overweight ≥25 - < 30 kg/m^2	0.31 (-3.03 - 3.64)	0.86
Normal weight < 25 kg/m^2	-	-
	N = 1910	
Perceived stress[4]	-0.08 (-0.35 - 0.19)	0.57
	N = 1655	
Educational level[4]**:**		
> 4 years higher education	3.71 (-1.60 - 9.02)	0.17
3 or 4 years higher education	3.97 (0.01 - 7.94)	**0.05**
< 3 years higher education	0.92 (-3.39 - 5.25)	0.68
Skilled worker or < 1 year higher education	-	-
	N = 1937	
Duration of the cold pressor test[4]**:**		
Completion of 1 < min	6.24 (2.29 - 10.19)	**0.002**
Completion of 1-2 min	3.06 (-0.56 - 6.68)	0.09
Completion of 2 min	-	-
VAS score of the cold pressor test[4]	1.03 (0.34 - 1.71)	**0.003**

[1]Unstandardized coefficients.
[2] All analyses were adjusted for baseline PPT, self-reported pain and use of pain medication. In addition, analyses on BMI, educational level, perceived stress, cold pressor test duration and VAS score were adjusted for sex and age.
[3] No significant interactions were found between sex and age (P = 0.34).
[4] No significant interactions were found between sex and: BMI (P =0.79), perceived stress (P = 0.78), educational level (P = 0.16) or cold pressor VAS score (P = 0.79). A significant interaction was found between sex and cold pressor test duration (P = 0.02).

Conditioned pain modulation and pressure pain sensitivity in the adult Danish general population: The DanFunD study

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Abstract

Increased pressure pain sensitivity and impaired descending pain control have been associated with chronic pain, but knowledge on the variability in the adult general population is lacking.

Pressure pain thresholds (PPTs) and descending pain control as assessed by conditioning pain modulation (CPM) were recorded in a randomly selected sample (n=2199, 53% females) of the Danish adult general population aged 18-70 years. PPTs were recorded over the tibialis anterior muscle and the upper trapezius muscle. CPM was defined as the difference between PPT assessments before and during conditioning with cold pressor pain (hand) for 2 min. Conditioning pain intensity was assessed on a visual analogue scale (VAS) and questionnaire data was collected.

Female sex ($P<0.001$) and younger age ($P\leq0.02$) was associated with lower PPTs at both body sites. For the trapezius muscle, high perceived stress were associated with lower PPTs ($P<0.02$), whereas an interaction was found between body mass index and sex. CPM potency was lower in females compared with males ($P\leq0.003$) whereas no association with age was found. Higher education ($P\leq0.05$), premature withdrawal from the cold pressor test ($P\leq0.02$) and high VAS score ($P\leq0.02$) were associated with a larger CPM response.

Perspectives

Data from this large population-based study provides new insight into the gender and age variation in pain sensitivity and CPM response. Decreased CPM potency and increased pain sensitivity in females were found, emphasizing the need to improve the understanding of its clinical consequences.

Introduction

Pain sensitivity and the function of descending pain control have been extensively studied in clinical populations [28, 43], but the variability in these pain measures using data from a large epidemiological study of the adult general population has not been published previously.

The status of specific pain mechanisms can be assessed experimentally by standardized activation of different pathways in the nociceptive system and quantitative assessment of the evoked responses [1, 2, 5, 53]. The conditioned pain modulation paradigm (CPM) is believed to reflect the net sum of descending pain inhibition and facilitation [47]. Assessment typically involves application of a conditioning tonic pain stimulus and probing the effect with a painful phasic test stimuli applied to an extra-segmental site [54]. Although CPM protocols vary across studies, consistent findings have shown decreased potency in chronic pain patients [17, 28, 31], but so far little is known about the variation of CPM in an adult general population. A systematic review based on 17 studies including 670 healthy participants in the reproductive age found that females show less efficient CPM compared with males, which has been suggested as an important factor in the higher prevalence of chronic pain found in females [37]. So far the difference in CPM between males and females has not been studied across the age span. Greater sensitivity to pressure pain stimuli has also been consistently observed in females compared with males [39], but the link between increased baseline pain sensitivity in females and the less efficient CPM response has so far not been examined [37]. While inconsistent results on the association between age and pain sensitivity have been reported [11, 14, 19] , previous findings on CPM efficacy in selected healthy adults suggest that there is an age related decline [12, 50] starting at middle-age (40-55 years) [24]. Studies examining the association between CPM potency, pain sensitivity and other health related factors that may influence the pain response have so far been inconclusive. Accumulating evidence point to an association between increased body mass index (BMI) and chronic pain [21, 32, 36, 51] and an influence on pain thresholds

has also been reported [38]. Sensitization of the nociceptive system due to systemic inflammation caused by fat tissue has been suggested as one possible explanation for this relationship [36]. BMI has so far not been examined in relation to CPM potency. Psychology has also been shown to influence the response to experimental pain [23, 44]. However, in relation to CPM a recent meta-analysis examining the potential confounding effects of psychological factors, e.g. perceived stress, concluded that results are inconclusive and that more research is needed [30]. Findings on the relation between the duration of the conditioning stimulus and CPM magnitude have so far been inconsistent in healthy subjects [15, 26, 40]. The influence of educational level on CPM and pain sensitivity has not been studied, but since the experience of pain is influenced by cognitively driven, supra spinal mechanisms the examination of a possible association is relevant [5, 56]. Moreover, an association between socio-economic factors and chronic pain is well established [10, 20]. Using an epidemiological approach, the primary purpose of the present study was thus to examine the sex and age related variations in CPM potency and pain sensitivity in the adult general population and secondly to examine the associations with BMI, level of perceived stress within the past month and educational level.

Methods

Study population

The present study is based on the first 2199 participants in the Danish Study of Functional Disorders (DanFunD). All invited persons were randomly drawn from the Danish Civil Registration system (each citizen in Denmark has a unique personal registration number), were between 18 - 70 years of age, and living in 10 municipalities in the south-western part of suburban Copenhagen. Exclusion criteria were: Not born in Denmark, not being a Danish citizen or pregnancy. The study was initiated November 2012 and pain assessment of participants was terminated by the end of 2013. Altogether 7942 were invited and 2199 participated (27.7%). All participants were fasting at the time of testing, i.e. no food or drinks after 11 pm prior to the day of testing. If a participant was scheduled for a time after 12.30 pm, a small meal no later than 6 hours prior to testing was allowed. Demographic and questionnaire data (i.e. self-reported pain, educational level and perceived stress) were collected. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). Information on use of pain medication either prescribed or over-the-counter drugs were registered on the day of testing. All participants gave written informed consent before taking part in the study, which was approved by the ethics committee (H-3-2012-015) and performed according to the principles of the Helsinki declaration.

Protocol

Participants were asked to lie down on a bed in a quiet room with the head elevated. Verbal information about the pain testing procedure was provided by a member of the staff performing the test. Participants were informed about the purpose of the study, pain testing procedures, and were asked to pay full attention to the procedure during the entire test. All 6 staff members performing the pain tests had received formal training in the testing procedures, which have all been validated

in other settings with good to excellent reliability [6 27]. The test procedure was as follows: 1) PPTs were assessed over the tibialis anterior muscle, 10 cm distal to the apex patellae on the non-dominant side and over the upper trapezius muscle, 10 cm from the acromion in direct line with the neck at the non-dominant side, 2) two minutes of cold pressor stimulation, and 3) re-assessment of PPTs over the tibialis anterior muscle during the cold pressor test.

Pressure algometry

PPTs at both testing sites were assessed with a handheld pressure algometer (Somedic, Sweden) mounted with a 1 cm^2 probe. The rate of pressure increase was kept at approximately 30 kPa/s. Participants were instructed to press a handheld push button to stop the pressure stimulation when the pressure sensation became painful, which defined the PPT. Participants were particularly instructed not to attempt to endure the pain as the test was a threshold and not a tolerance measure. A training procedure was applied in order to familiarize the participants with the assessment conditions. After the training, three PPT assessments were completed with 20 s intervals, never applying the algometer on the same skin spot although in close proximity. The mean value of the three PPTs recordings defined the PPT for further analysis.

Cold pressor stimulation and conditioned pain modulation

The participants immersed their dominant hand (to the wrist) in a circulating water bath at a cold temperature (maximum 3 °C) for 2 minutes. The water temperature was checked prior to each test to ensure that it was in accordance with the protocol. PPTs on the tibialis anterior muscle were reassessed (mean of three) after 1 minute of hand immersion following the same procedure as at baseline. If the conditioning pain stimulus became intolerable, the participant could terminate the stimulation earlier than scheduled. Participants were instructed to give notice before withdrawing

the hand so an assessment of PPT could be performed. The premature withdrawal in some individuals implied that PPT measurement was initiated before 1 minute of hand immersion. Data was still included in the statistical analyses in case only one or two of the three PPT recordings were obtained. Changes in PPTs from baseline to the reassessments during the conditioning cold-pressor stimulation were considered to reflect the CPM effect. The CPM effect was determined both as the absolute and percentage change as recommended by Yarnitsky and colleagues [53, 54]. After withdrawing the hand from the water bath, participants rated the intensity of the pain experience on a Visual Analogue Scale (VAS) where a score of 0 cm reflected "no pain" and a score of 10 cm reflected "worst pain imaginable".

Questionnaire data

Self-reported pain: Assessment of pain symptoms was based on a questionnaire about the presence of pain from muscles or joints, back pain, pain in the extremities, headache, chest or stomach pain within the past 12 months. Degree of pain was rated on a 5-point Likert scale with the following categories: "*not at all*", "*a little", "some", "a great deal" and "very much*". Scores were summarized into an ordinal scale ranging from 0 (no pain) to 28.

Educational level: Level of education was classified into 4 groups and defined as: 1) Skilled worker or less than 1 year of higher education, 2) less than 3 years of higher education, 3) 3 or 4 years of higher education, and 4) more than 4 years higher education.

Perceived stress: The Perceived Stress Scale (PSS) contains questions about thoughts and feelings during the past month and measures the degree to which situations in a person's life are appraised as stressful [7]. The PSS has been adapted to a short version consisting of 10 questions, the PSS-10, which has proven to be a valid and reliable measure of perceived stress [8]. Responses on the PSS-10 are rated on a 5-point Likert scale with the following categories: *"never", "almost never",*

"sometimes", "fairly often", and "very often". If more than 5 items were missing, the score was not included in the statistical analyses. The Danish translation has been validated by back translation to the original language and approval by the developer of the scale [33].

Statistics

Statistical analyses were performed using SPSS for Windows (version 22). Skewness and kurtosis and visual inspection of Normal Q-Q plots were used to inspect normality. Descriptive statistics are presented as mean and standard deviations (SD) or median and interquartile range (IQR) depending on the distribution of the continuous variables or as frequencies for categorical variables. Independent samples t-test, Pearson Chi-square, and Mann-Whitney U tests were used to examine differences in the distribution of age, BMI, perceived stress, educational level, self-reported pain symptoms (Likert scale) and use of pain medication (yes/no) between males and females. Level of significance was set at $P < 0.05$.

Baseline PPTs: With the purpose of testing the associations between PPTs at the tibialis anterior muscle and upper trapezius muscle and sex, age, BMI, perceived stress and educational level, multiple linear regression analyses were performed with PPTs as the dependent variables and the remaining variables as the explanatory variables. Associations of PPTs with sex and age, respectively, were examined in the first model adjusting for self-reported pain and use of pain medication. Next, BMI, perceived stress and educational level were analyzed in separate regression models adjusting for sex, age, self-reported pain and use of pain medication. Due to non-normal distributions, the PPT variables were log transformed prior to statistical analysis. The back transformed estimates and 95% confidence interval (CI) are presented. Initial analyses showed a quadratic rather than linear relationship between PPTs and age ($P < 0.01$), and age was thus categorized into four groups (18-39, 40-49, 50-59, 60-70 years). The same applied to the

relationship between PPT at the upper trapezius muscle and BMI, and the BMI variable was thus categorized according to the WHO recommendations [9]. Since the group with a BMI less than 18.5 kg/m^2 was rather small (N = 60), this group was merged with the normal weight group (BMI: 18.50 - 24.99 kg/m^2).

CPM effect: To analyze the association between the CPM effect, sex and age a multiple linear regression analysis was applied with the CPM effect as the dependent variable and sex and age as the explanatory variables. Since the CPM effect is relative to the baseline PPT value, controlling for baseline PPT value is relevant when examining the association between CPM and other factors. Associations with BMI, educational level, perceived stress, VAS score and duration of the cold pressor test were subsequently analyzed in separate linear regression models after adjusting for baseline PPT, sex, age, self-reported pain and use of pain medication. A total of 748 (38.9%) completed the cold pressor test for 2 min and the variable was thus categorized into 3 groups for the statistical analyses: 1) completion of 2 min, 2) completion between 1 min and less than 2 min, and 3) completion of less than 1 min. Subsequent sensitivity analyses were performed for the cold pressor test variable including sex, age, baseline PPT, self-reported pain and pain medication use in the model.

To check if inclusion of data based on less than 3 PPT assessments influenced the CPM findings, a control analysis on sex and age adjusted for baseline PPT, self-reported pain and medication use was performed including only participants with triple PPT recordings.

Results

Demographic characteristics

Compared to non-respondents, more females than males took part in the study ($P < 0.001$) and mean age was higher in respondents (50.7 (sd 13.5) years) compared to non-respondents (44.7 (sd 15.9) years ($P < 0.001$)). In this study, more females than males participated whereas no difference in mean age was observed between sexes (Table 1). Self-reported pain and use of pain medication were more frequent in females than in males. The same applied to level of perceived stress that was higher in females, whereas males had significantly higher BMI and levels of education (Table 1). Figure 1 shows the number of participants for each pain assessment procedure.

Baseline pressure pain sensitivity

The tibialis anterior muscle: The median (IQR) PPT at the tibialis anterior muscle in the total population was 512.0 kPa (329.3 kPa). Examining baseline PPT in participants with only complete PPT measurements (i.e. 3 assessments) did not change the results (median (IQR): 512.0 kPa (329.0 kPa)). Descriptive statistics of baseline PPT in males and females across age groups, BMI and educational level are presented in Table 2. The linear regression analysis demonstrated that compared with males, females had lower PPTs and the youngest age group had lower PPTs compared with the age groups above 40 years (Table 3). No associations were found with BMI, perceived stress or educational level (Table 3).

The upper trapezius muscle: The median (IQR) PPT at the upper trapezius muscle in the total population was 446.3 kPa (275.3 kPa). Examining baseline PPT in participants with only complete PPT measurements (i.e. 3 assessments) did not change the results (median (IQR): 446.0 kPa (275.0

kPa)). The linear regression analysis showed that females had lower PPTs compared with males, and the younger age groups had lower PPTs when compared to the older age groups above 50 years (Table 3). A significant interaction was found between sex and BMI suggesting that the effect of sex on PPT differed across BMI levels. High perceived stress was associated with lower PPTs whereas no association with educational level was found (Table 3).

Conditioning pain modulation

In the total sample the duration (median/IQR) of the conditioning cold pressor stimuli was 106.0 (65.0) s; 117.0 (40.5) s in males compared to 98.0 (70.0) s in females ($P < 0.001$, Mann-Whitney U Test). The conditioning VAS score was (median/IQR) 7.0 (3.0) cm in the total sample, and 6.0 (3.0) cm in males compared to 7.0 (3.0) cm in females ($P < 0.001$, Mann-Whitney U Test).

Scatterplots of raw PPT and CPM data in males and females across age groups are shown in Figure 2. In the total population, the absolute CPM effect was (median (IQR)) 178.0 kPa (190.1 kPa) and the relative increase was 35.9 % (43.2 %). Descriptive statistics of the CPM in males and females across age groups, educational level and BMI are shown in Table 4. The CPM effect was larger in males than in females after adjusting for baseline PPT both in absolute terms (Table 5) and in relative terms (Table 6). No significant linear relationship between the CPM effect and age was found (Table 5 and Table 6). Including age as a continuous variable in the model (absolute CPM) instead of the categorical variable did not change the result ($P = 0.69$). No associations with BMI or perceived stress was found, whereas a positive linear relationship (i.e. a large CPM effect) with increasing educational levels was found although only significant when having 3 or 4 years of higher education. A significant positive linear relationship with increasing cold pressor VAS score was found. Completing less than 2 min of the cold pressor test was also associated with a higher

absolute CPM response (Table 5), whereas the same applied for completing less than 1 min for percent CPM.

Performing sensitivity analyses in relation to the cold pressor test including only the participants who completed the per protocol 2 min of cold pressor stimulation (n = 772) and the participants who completed less than 1 min (n= 477) showed that in the group that completed the 2 min with cold pressor test, the absolute (P = 0.11) and percent difference (P = 0.28) in CPM between males and females was no longer significant, whereas the results for age remained unchanged ($P \geq 0.10$). In the group that completed less than 1 min, a significant effect of sex was confirmed with the magnitude of the CPM response being smaller in females compared with males (absolute CPM: $P < 0.001$, percent CPM: $P < 0.001$), whereas no significant effect of age was found ($P \geq 0.61$). Examining the distribution between males and females in the two groups showed a significant difference with a preponderance of males completing the 2 min compared with females, and a preponderance of females in the group completing less than 1 min ($P < 0.001$).

Performing control analyses on sex and age including only participants with three complete PPT assessments in relation to the cold pressor test (n = 1227) showed the same tendencies (Sex: P = 0.02 (absolute CPM) and 0.045 (percent CPM), Age: $P \geq 0.34$ (absolute CPM) and $P \geq 0.34$ (percent CPM) as reported in Table 5 and 6, i.e. the CPM response was larger in males compared with females and there was no association with age.

Discussion

Data from this large, randomly selected sample of the adult general population confirmed that females were more sensitive to pressure pain as compared with males and had reduced CPM potency. Younger age was associated with being more sensitive to pressure pain, whereas no association between age and CPM was shown. High perceived stress was associated with lower trapezius PPTs, but not with PPTs at the tibialis or CPM. The same applied to BMI, where an interaction with sex was found. No associations between educational level and pressure pain sensitivity was found, whereas having 3 or 4 years of higher education was associated with a larger CPM response. Premature withdrawal from the cold pressor test and high VAS score were both associated with a larger CPM response.

Pressure pain sensitivity

Previous findings on the association between sex and pain sensitivity in nonclinical populations have been inconsistent, which may in part be explained by the heterogeneity of available methods for evaluating the pain response, study populations and insufficient statistical power in some studies [39]. However, the present data support the conclusions based on systematic reviews that point to greater pain sensitivity in females compared with males and especially to pressure pain assessments [3, 29, 39]. The factors, whether physiological, psychological or psychosocial or an interplay, responsible for this difference have so far not been fully clarified [3, 20], although some studies suggest that gender-specific psychological factors influence the response to pain and thus offer some explanation to the observed male-female differences [23, 44].

The results also support existing studies on age and PPTs pointing to increased thresholds with advancing age [22, 24, 25]. For example, Jensen and colleagues examined PPTs in a random sample of

1000 adults aged between 25 - 64 years [22], and found significantly increasing thresholds with advancing age. Lariviére and colleagues [24] examined 60 healthy adults and likewise found lower PPTs in the youngest participants compared to both middle-aged and older participants. Some authors have suggested that findings of age differences in pain thresholds may depend on stimulus duration and that a longer response time in older people may account for the differences [19]. Although results from this study is limited to PPT assessments, no differences in PPTs between the age groups above 40 years for assessments on the tibialis anterior muscle and above 50 years for assessments on the upper trapezius muscle were found suggesting that a longer response time in the elderly have not influenced the findings.

High perceived stress was associated with lower PPTs, but only at the upper trapezius muscle. Increased sensitivity to pressure pain at the trapezius muscle has been reported in a study comparing persons on sick leave due to stress with healthy controls [18], and it is thus likely that high levels of perceived stress may contribute to increased pain in this particular body area [52]. Being obese or overweight was associated with higher PPTs (i.e. less pain sensitivity) at the upper trapezius muscle whereas no association was found for the tibialis anterior muscle. However, a significant interaction between sex and BMI was found for the upper trapezius muscle suggesting that the strength of the linear relationship across BMI levels differ between males and females. A recent systematic review concluded that existing studies do not clearly demonstrate that body weight influence pain perception [45]. One study has reported site-specific differences in pain sensitivity in obese and non-obese persons between 18 and 45 years of age with the obese being less sensitive, but only in areas with excess subcutaneous fat, e.g. the abdomen [38]. In support of this conclusion, the study found no differences in central pain processing, i.e. temporal pain summation and CPM [38]. In line, no association between BMI and CPM was found in the present study. Nevertheless the present data altogether suggests that the upper trapezius is a body area more sensitive to pain stimulation than

the tibialis anterior as shown by the lower overall thresholds levels in both males and females and the influence of perceived stress and BMI.

Conditioned pain modulation

Significant differences in CPM potency were found between males and females with the response being smaller in females after adjusting for baseline pain sensitivity, self-reported pain and use of pain medication. Inconsistent findings on sex-related differences in CPM have been reported in more studies with some reporting no observed difference [34, 35, 46, 49]. However, a systematic review concluded that existing data support a decreased CPM response in females compared with males, and in particular in studies using pressure pain stimulation [37]. A recent study assessing relative CPM by utilizing pressure pain thresholds and a cold pressor test in a group of healthy university students further support this conclusion [4]. Though a major strength of this study is the large sample size, the difference between males and females was no longer significant when examining the subgroup that completed the per protocol 2 min. This finding could suggest that sensitivity to the cold pressor test constitute a bias when applied as a conditioning stimulus. Premature withdrawal from the cold pressor test and higher pain intensity scores were both associated with a larger CPM response, which additionally suggest that both sensitivity and the subjective intensity of the cold pressor test plays a role in the potency of the CPM response. A number of studies on the involvement of intensity and duration of the conditioning stimulus on CPM extent have been performed [15, 26, 40]. These studies suggest that the CPM response is not dependent on conditioning stimulus intensity, i.e. painful versus non-painful [26], pain intensity [15, 55] or duration [40]. While not the primary aim, others have reported that CPM effect do in fact depend on, e.g. the intensity of the conditioning stimuli [13]. Altogether, the present study support a male-female difference in CPM

potency, but this finding needs to be replicated in another epidemiological study applying a different CPM methodology before any firm conclusions can be drawn.

The effects of aging on the CPM response have been tested in healthy individuals in previous studies using different methodologies [12, 16, 24, 41, 42, 50]. Although findings from these studies are inconsistent and the study populations are generally small, the majority report an age-dependent decline in CPM and are, therefore, in contrast to the present study. However, this study is based on a large random sample of the general population and all analyses were adjusted for baseline PPT, self-reported pain and use of pain medication which supports the findings of no association between age and CPM magnitude. Nevertheless, further studies are needed to determine the role of age in CPM.

A significant association was found between CPM and educational level, i.e. the CPM effect was larger in participants with 3 or 4 years of higher education compared to being a skilled worker or having less than 1 year of higher education. No other significant associations were found and comparisons with other studies are not possible since no other studies have examined this relationship. Moreover, no associations between PPTs and education were found. However, while the effect of education was small in this study other research has shown that education may influence disease status [48] and further exploration is thus needed.

Strengths and limitations

A major strength of this study is the population-based design including a random sample of the adult general population, but some limitations must be considered when interpreting the findings. The study was conducted in a random sample of the Danish adult general population and the results may thus not necessarily be generalizable to other populations. The response proportion of 27% may be considered low and with a possible influence on the results because of selection bias.

Comparing respondents with non-respondents showed that females were more likely to participate in the study compared with males and older people more likely than younger, which may affect the generalizability of the results.

Self-reported pain and use of pain medication were included in the analyses as possible confounders. However, some limitations must be considered since the duration of self-reported pain, i.e. whether acute/subacute or chronic, could not be determined based on the pain questionnaire data used in the present study, which enquired about the presence and intensity of pain during the past 12 months. Furthermore, participants may have experienced pain from other parts of the body than the ones enquired about.

Altogether, only 39% of the study population completed the cold pressor test according to the protocol, which could suggest that the conditioning stimulus were perceived as strong by the majority. Although no specific CPM protocol has been recommended [54] reducing the time of the conditioning stimulus, i.e. the cold pressor test, or applying individually tailored temperature levels may improve compliance with the testing procedure and thus reduce the possibility of bias. However, variations in the ability or willingness to undergo a painful testing procedure are to be expected in a general population sample despite careful instructions from the staff performing the tests.

Conclusions

This study provides new knowledge on the gender and age variation in pain sensitivity and descending pain control by using data from a large and randomly selected adult population-based sample. The confirmation of decreased CPM potency and greater pain sensitivity in females compared with males emphasize the need to improve the understanding of the clinical consequences

in females and thus more research into the mechanisms responsible for the male-female difference. Younger age was associated with greater pain sensitivity, whereas an age-dependent decline in CPM potency could not be confirmed and age may thus not play a major role in the individual ability to modulate pain. The trapezius area was more sensitive to pressure pain than the tibialis and an association with high perceived stress was found, which suggests that psychological factors needs to be taken into account. Duration and intensity of the conditioning stimulus was associated with a larger CPM response suggesting that CPM is not entirely independent of methodology.

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Author contributions

SS, TJ, LAN, JFE, and TGN conceived and designed the study protocol for the present study, interpreted the data, and critically revised the manuscript. SS analyzed the data and drafted the manuscript. As PI and member, respectively, of the DanFunD steering committee TJ and SS designed and initiated the DanFunD study and JFE contributed to all parts of the process as well as being the daily project manager. TC contributed to the design of the DanFunD study and critically revised the manuscript. All authors discussed the results and commented on the manuscript and approved the final version.

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Figure 1: Participant flowchart for the pain testing sequence

Figure 2AB: Scatterplot of raw data on baseline PPT and CPM in males (A) and females (B)

Tables:

Table 1: Sample characteristics

Table 2: Descriptive statistics of PPTs across sex, age groups, BMI and educational level.

Table 3: Multiple linear regression analyses with PPTs as the dependent variables and sex, age, BMI, educational level and perceived stress as the explanatory variables.

Table 4: Descriptive statistics of CPM across sex, age groups, BMI and educational level presented as medians (IQR).

Table 5: Multiple linear regression analyses with absolute CPM as the dependent variable and sex, age, BMI perceived stress, educational level, cold pressor test duration and VAS score as the explanatory variables.

Table 6: Multiple linear regression analyses with percent CPM as the dependent variable and sex, age, BMI perceived stress, educational level, cold pressor test duration and VAS score as the explanatory variables.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page # where this item is located:
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3 - 4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5 - 8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the	

		number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8 - 9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5 - 8
Bias	9	Describe any efforts to address potential sources of bias	8 - 9
Study size	10	Explain how the study size was arrived at	Not relevant

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8 - 9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8 - 9
		(*b*) Describe any methods used to examine subgroups and interactions	8 - 9
		(*c*) Explain how missing data were addressed	8 - 9
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	8 - 9
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1
		(b) Give reasons for non-participation at each stage	Figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1, 2 and 4
		(b) Indicate number of participants with missing data for each variable of interest	Table 2 and 4
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	Not relevant
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 3 and 5, page 10 - 12

	(*b*) Report category boundaries when continuous variables were categorized	8 - 9
	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Table 3 and 5, page 12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16 and 17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13 - 18
Generalisability	21	Discuss the generalisability (external validity) of the study results	16 and 17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18